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MIPR NO: 94MM4563

TITLE: Predictive Value of Serum Organochlorine Levels and Breast Cancer in Occupationally Exposed Populations

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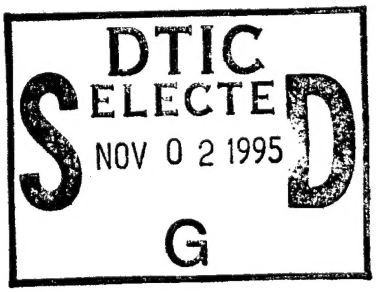
REPORT DATE: October 1995

TYPE OF REPORT: Annual



PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1995	Annual 13 Jun 94 - 30 Sep 95

4. TITLE AND SUBTITLE
Predictive Value of Serum Organochlorine Levels and Breast Cancer in Occupationally Exposed Populations

5. FUNDING NUMBERS
94MM4563

6. AUTHOR(S)
Paul A. Schulte

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
National Institute for Occupational Safety and Health
Cincinnati, Ohio 45226

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Exposures to organochlorine compounds have been suggested as risk factors for breast cancer because of their estrogen-like effect. The objective of this study is to determine if the high serum levels of various organochlorine compounds, detected 5-20 years before diagnosis, are risk factors for breast cancer, utilizing serums collected by the Janus Bank prior to diagnosis. Since we identified too few subjects in high risk occupations and there was concern about over matching, we chose to study employed women (including farmers wives) that also includes the high risk occupations. The original plan was to analyze the estrogen and anti-estrogen compounds on separate groups of cases and controls. However, protocol reviewers suggested that the study would be stronger if all compounds were measured on the same subjects. A selection algorithm has been developed, 150 cases and 150 matched controls have been selected and specimens will be delivered to the CDC on September 26th, 1995. Since only 1.1 ml of serum are available on each subject, the laboratory has been developing methods to assay both types of compounds on this amount of serum. Final tests are currently underway to see if the assays can measure these substances on 1.1 ml samples.

14. SUBJECT TERMS
Organochlorine, breast cancer, Janus Bank, case-control, control selection algorithm, anti-estrogens, estrogens

15. NUMBER OF PAGES
11

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

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Paul A Schulte 9-1-95

PI - Signature Date

Table of Contents

Introduction

The role of organochlorine compounds in breast cancer is still uncertain. It is not known whether estrogens act as initiators, promoters, or both with regard to breast cancer. Moreover, the diversity of estrogenic chemicals is quite large and estrogenic response in breast tissue is a complex process that is yet to be elucidated. However despite these unknowns, the circumstantial evidence that estrogens and estrogen-like compounds in some way contribute to risk for breast cancer is compelling. Our study has the potential to contribute useful information to the question of the role of estrogenic agents in breast cancer risk by evaluating separately and simultaneously more than 30 organochlorine compounds, some of which are estrogenic and some are antiestrogenic. We had originally planned to conduct two related case-control studies, one evaluating estrogenic pesticides and PCBs and the other evaluating dioxins, furans, and PCBs that have antiestrogenic properties. Now, for a variety of reasons, primarily because it is a stronger design, we hope to conduct the study in one case-control design where both estrogenic and antiestrogen compounds will be evaluated in the same subjects.

Body

At present, the preparatory steps prior to sample analysis have been performed. These steps include:

1) **Planning sessions with the Janus Serum Bank and the Norwegian Cancer Registry.**

Drs. Schulte and Friedland went to Norway in August 1994 to meet with

Dr. Egil Jellum of the Janus Bank and Dr. Aage Andersen of the Norwegian Cancer Registry and their staff to plan scientific and logistical aspects of the study. Planning has continued since that meeting.

2) **Review of study protocol.**

The initial study protocol was sent to Dr. Nancy Krieger (Kaiser Foundation) and Dr. Mary Wolff (Mt. Sinai) for review. Their comments have been considered.

3) **Revision of study design.**

Based on enhancement of the analytic procedure, comments of the reviewers, and the fact that there would not be enough cases in the Janus Serum Bank that meet the initial selection criteria, the study design was revised. A stronger design, which allowed for the measurement of estrogenic and antiestrogenic compounds on the same person, was adopted.

The protocol originally called for 276 cases and 276 controls for a total of 552 assays. The modified protocol called for 150 cases and 150 controls for a total of 600 assays. Our laboratory collaborators have agreed to do this. Thus, instead of a total of 552 subjects from the Janus Bank, we will only receive 300, plus the few (5 or 10) extra representative samples for testing the method. The revision was communicated to Mr. Craig Lebo, U.S. Army Medical Research Command, and approval was received dated 4/7/95.

4) **Development and implementation of subject selection algorithm.**

The subject selection strategy and algorithm has been developed. In the process, the strategy was sent to Dr. Sholom Wacholder (NCI) and Dr. Kyle Steenland at NIOSH for review. Initial efforts to restrict the study groups to high-risk occupations was rejected due to concerns about over-matching and too few potential subjects. The strategy now focuses on employed women including farmers wives. A copy of the selection strategy is attached.

Computer specialists and epidemiologists at the Norwegian Cancer Registry have applying the selection strategy to the population of subjects in the Janus Bank and in conjunction with staff from the Janus Bank have identified and retrieved all the study specimens from the Janus Bank. Samples will be delivered to the CDC on September 26 th.

5) **Enhancement of the analytic approach.**

Laboratory tests were conducted to determine whether it is possible to measure more than 30 estrogenic and antiestrogenic organochlorines on 1.1 ml of blood. Preliminary indications are that it is possible and the methods are being refined. A batch of (nonstudy) specimens from the Janus Bank representing specimens taken during the same time periods as study specimens was sent to the laboratory of the National Center for Environmental Health in Atlanta. These specimens will be used to determine whether the analytical methods are capable of identifying organochlorines in the range of concentrations found in these specimens. The batch of specimens also allowed us to successfully demonstrate that

we could actually ship specimens frozen and have them arrive frozen.

At present, we believe we will adhere to the original schedule for analysis in the initial protocol. Thus, 50% of the samples should be analyzed by February 1996. If, however, there is a prolonged government shutdown, this schedule will need to be revised. After the specimen analysis efforts are underway, the focus will be to develop a statistical analysis plan for the data.

Conclusion

The study has been slowed due to revisions and problems encountered, but we believe that the resultant product will be stronger. Barring any delays, we expect to finish the study exactly on schedule.

References

Davis DL, Bradlow HL, Wolff M, Woodruff T, Hoel DG, Anton-Culver H: Medical hypothesis: xenoestrogens as preventable causes of breast cancer. Env Health Persp 101: 372-77, 1993.

Sternberg SS: Re: DDT and breast cancer. JNCI 86: 1094, 1994.

Krieger N: Re: DDT and breast cancer. JNCI 86: 1094-5, 1994.

Wolff MS, Toniolo PG: Re: DDT and breast cancer. JNCI 86: 1095-96, 1994.

Colburn T, Vom Saal FS, Solo AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Env Health Persp 378-89, 1993.

Gaard M, Tretli S, Urdal P: Risk of breast cancer in relation to blood lipids: a prospective study of 31, 209 Norwegian women. Cancer Causes and Control 5: 501-09, 1994.

Dewailly E, Ayotte P, Brisson J, Sylvie Dodin. Breast cancer and organochlorines. Lancet 344: 1707-8, 1994.

Jellum E, Anderson A, Lund-Larsen P, Theoclorsen L, Orgasaeter H: Experiences of the Janus Serum Bank in NOrway. Env Health Persp 103(suppl 3) 85-88, 1995.

Svensson BG, Hallberg T, Nilsson H, Schutz A, Hagmor L: Parameters of immunological competence in subjects with high consumption of fish contaminated with persistent organochlorine compounds. Int Occ Env Health 65: 351-58, 1994.

Case-Control Study of Serum Organochlorines and Breast Cancer

Subject Selection

This is a matched case-control design with restriction of subjects on gender and employment.

1. Subjects (cases and controls) will be selected from the Janus Serum Bank. Subjects will be restricted to those females in the Janus Bank who were employed according to the 1970 or 1980 census. Wives of farmers are considered as farmers, and hence employed and eligible for the study.

2. Cases will be selected if they meet the following eligibility requirement: There is at least 1.1 ml of serum collected at least two years before the date of breast cancer diagnosis.

3. For cases with multiple specimens, the first eligible specimen by date of collection will be selected.

4. Controls must be cancer free except for basal cell carcinoma of the skin and each control must be alive at the time of diagnosis of her matched control.

5. Matching controls to cases:
   Controls will be matched to cases on age at collection (± 2 years) and date of blood collection (± 2 years).

6. Procedure:

Since the eligibility of cases and controls is not known until each specimen is visually evaluated, the following multi-step procedure should be used.

1) Selection of cases:

Adhering to the restriction criteria, cases will be randomly selected from the pool of potential cases and the volume of the first serum specimen evaluated. If 1.1 ml is available, that person will be a case. Repeat this process until 150 cases are selected.

2) Selection of controls:

For each case, a control should be randomly selected from the subset of potential controls with the age (± 2 years) and date of collection (± 2 years). If 1.1 ml of blood is available and the control was alive at the time of diagnosis of the case, this woman will be a matched control. If 1.1 ml is not available, another random sample should be drawn from the subset of potential controls. This process should be repeated until controls are matched to all 150 cases.